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Light changes the membrane potential and ion balances of retinal rod disks

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Light stimulation of rod cells in vertebrate eyes may cause Ca^{2+} release from the intracellular disks. Radiolabelled tracers show that light causes a small hyperpolarization of intact disk stacks and redistribution of the ions Ca^{2+} and Cl^-.

Membrane potential *Vision* *Disk* *Calcium transport* *Ion transport* *Methyltriphenylphosphonium*

1. INTRODUCTION

The mechanism by which light absorbed by rhodopsin on the intracellular disk membranes of bovine retinal rod cells leads to a change in plasma membrane Na^+ permeability is not clear [1–11]. One possibility is that transport of ions (particularly Ca^{2+}) [12–16] across the disk membranes may generate one of the second messengers carrying the signal across the cytoplasm. Various groups have examined ion balances [17–26] and membrane potentials [27–31] in disks but no consensus has been reached on either the properties of the disk membrane or any changes which may occur in response to light.

Here we describe experiments designed to characterize: (a) the passive permeability of disk membranes to the ions Ca^{2+}, K^+, Na^+ and Cl^-; (b) the membane potential across the disk membrane; and (c) the changes in these parameters induced by light.

We find that light decreases $^{45}Ca^{2+}$ accumulation, increases $^{36}Cl^-$ accumulation and increases [^{3}H]methyltriphenylphosphonium ([^{3}H]TPMP$^+$) accumulation (a measure of membrane potential) in retinal rod disks incubated in a physiological medium.

2. MATERIALS AND METHODS

Rod outer segments (ROS) with over 85% broken plasma membranes were prepared as described in [32]. ROS were stored at 0–4°C in darkness in medium A (containing 90 mM K gluconate, 20 mM NaCl, 1 mM KH_2PO_4, 5 mM $MgSO_4$ and 10 mM Pipes, pH 7.4). ROS were incubated at 10–30 μM rhodopsin at 37°C in medium A with various suplements and accumulation of radiolabel was determined by centrifugation to pellet the ROS, and scintillation counting of supernatant and pellet. Accumulation ratios were calculated as (total internal ion content/intradiskal volume)/total external ion concentration, which takes no account of binding. As inulin can not cross the disk membrane the pellet content of radiolabel was corrected for trapped supernatant by [^{3}H]inulin content. In all experiments intradisk volumes were measured in parallel by the difference between ^3H_2O and [^{3}H]inulin spaces (see [33]). Volumes varied from day to day in the range 0.15–0.25 μl/nmol rhodopsin. This agrees with estimates from electron micrographs of our preparation but is about 5-times larger than in vivo. This swelling allows the experiments to be performed but may alter disk behaviour.

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3. RESULTS

Fig. 1 shows the time course of $TPMP^+$ accumulation into ROS in light and darkness. The equilibration of $TPMP^+$ is rapid. At early times the accumulation ratio of $TPMP^+$ was lower in darkness than in light. Does this accumulation represent binding of $TPMP^+$ to the membranes of ROS rather than a genuine accumulation ratio of free ion due to a transmembrane potential? Fig. 1

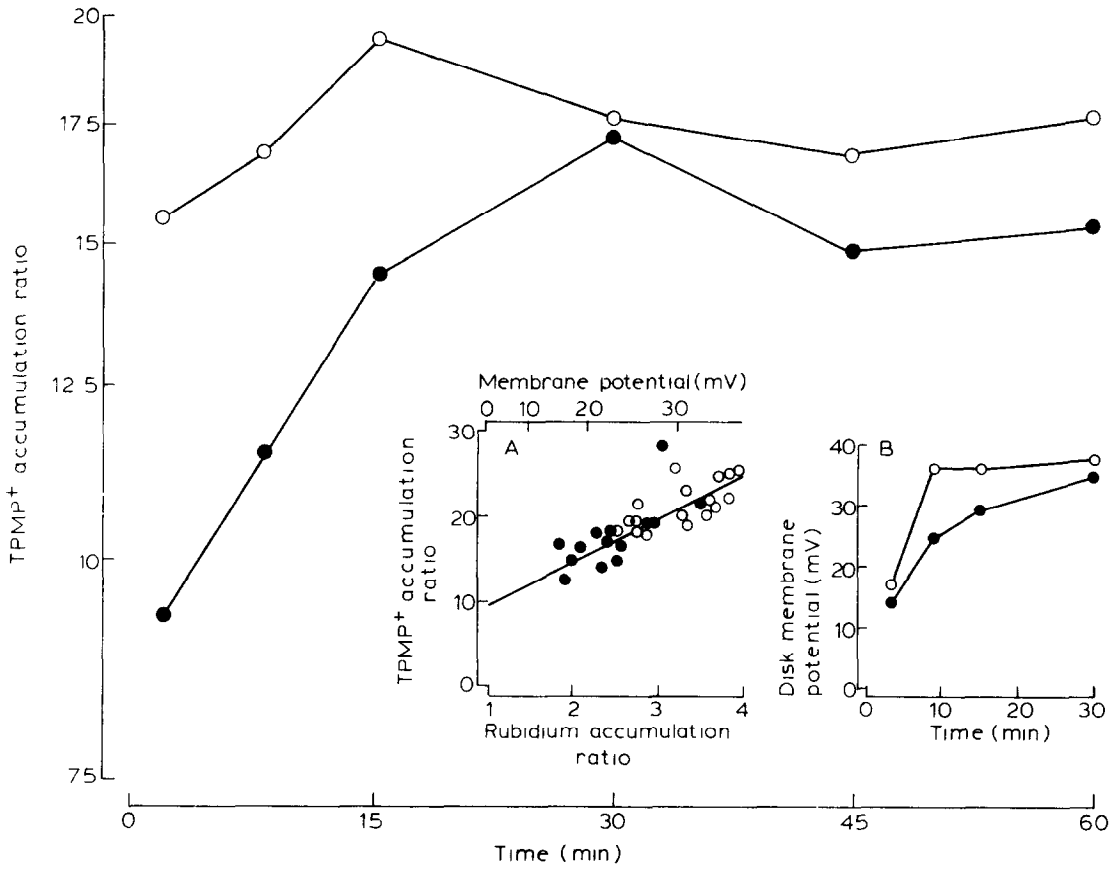

Fig.1. Disk membrane potential in darkness and light. ROS were incubated in 0.5 ml aliquots of medium A supplemented with 1 mM ATP, 0.5 mM GTP, 1 μM TPMCl, 0.1 mM inulin at 37°C in darkness (●—●) or fluorescent room light ($t_{1/2}$ bleaching = 15 s, ○—○). Samples were incubated for times indicated with either 2 μCi/ml [^{3}H]TPMPCl or 1–2 μCi/ml [^{3}H]inulin and then centrifuged at 11 000 × g for 1.5 min to pellet membrane material. Supernatant was withdrawn for scintillation counting, the pellet dried with a tissue, resuspended in H_2O and counted. After correction for supernatant trapped in the pellet by using the [^{3}H]inulin content the disk content of isotope was calculated [33]. In all experiments volumes were measured in parallel by replacing radiolabels with 0.6–1.5 μCi/ml ^3H_2O and again correcting for [^{3}H]inulin space. Points are means of triplicates on 5 preparations. Not all points have the same number of estimates. $TPMP^+$ accumulation ratio varied between experiments; see table 1 for a statistical assessment of data. (Inset A) Calibration of $TPMP^+$ accumulation ratio. Incubation was performed as above for 15 min in media with 2 μCi/ml [^{3}H]TPMPCl, 10 nM 0.2 μCi/ml ^{86}RbCl and 40 μM valinomycin (0.4% ethanol final) with Li^+ substituted for K^+ to give various K^+ concentrations in the range 1.91 mM. Light (○) appeared to increase disk K^+ content compared to darkness (●) but did not alter the relationship between $TPMP^+$ and Rb^+ accumulation ratios. Clamped V shown on the upper scale was measured by $^{86}Rb^+$ binding and calculated from the Nernst equation. These data are from one of the calibration curves used in calculating inset B and give a fair representation of the scatter in such an experiment. (Inset B) Membrane potential in disks. Incubations as for main figure. Points are averages of quadruplicates from 2 experiments calculated from the accumulation ratio of $TPMP^+$ using calibration curves such as inset A.

inset A shows the calibration of $TPMP^+$ accumulation against membrane potential (V), set by varying the K^+ concentration outside ROS in the presence of valinomycin, and measuring the clamped potential by $^{86}Rb^+$ distribution (which behaves similarly to K^+). The relationship between $TPMP^+$ accumulation and V was independent of light. It is clear that about 90% of $TPMP^+$ accumulation is due to binding to the membrane but this method of calibration of accumulation ratio vs known potential shows unambiguously that disks do have such a potential in the absence of valinomycin. The extent of binding varied from day to day so it was necessary to perform a calibration on every preparation to determine the value of V. Fig. 1 inset B shows the average of two such experiments. We do not regard the calibration as accurate, particularly at low potentials, but it is clear that the disks do have a membrane potential with a value of about 30 mV (positive outside). Whilst the light-exposed ROS have the potential at the earliest times examined the dark ones only develop it slowly.

Fig. 2 shows the accumulation of 4 ions into ROS under conditions identical to those in fig.1. Panels A and B show that the accumulation ratio of $^{86}Rb^+$ and $^{22}Na^+$ is about 1 when equilibration has occurred. It appears that K^+ (and $^{86}Rb^+$) are substantially more permeant than Na^+ particularly bearing in mind the 4.5-fold higher K^+ concentration outside the disks. This conclusion was supported by experiments examining the relative rate of swelling of disks in K^+ and Na^+-containing media (not shown).

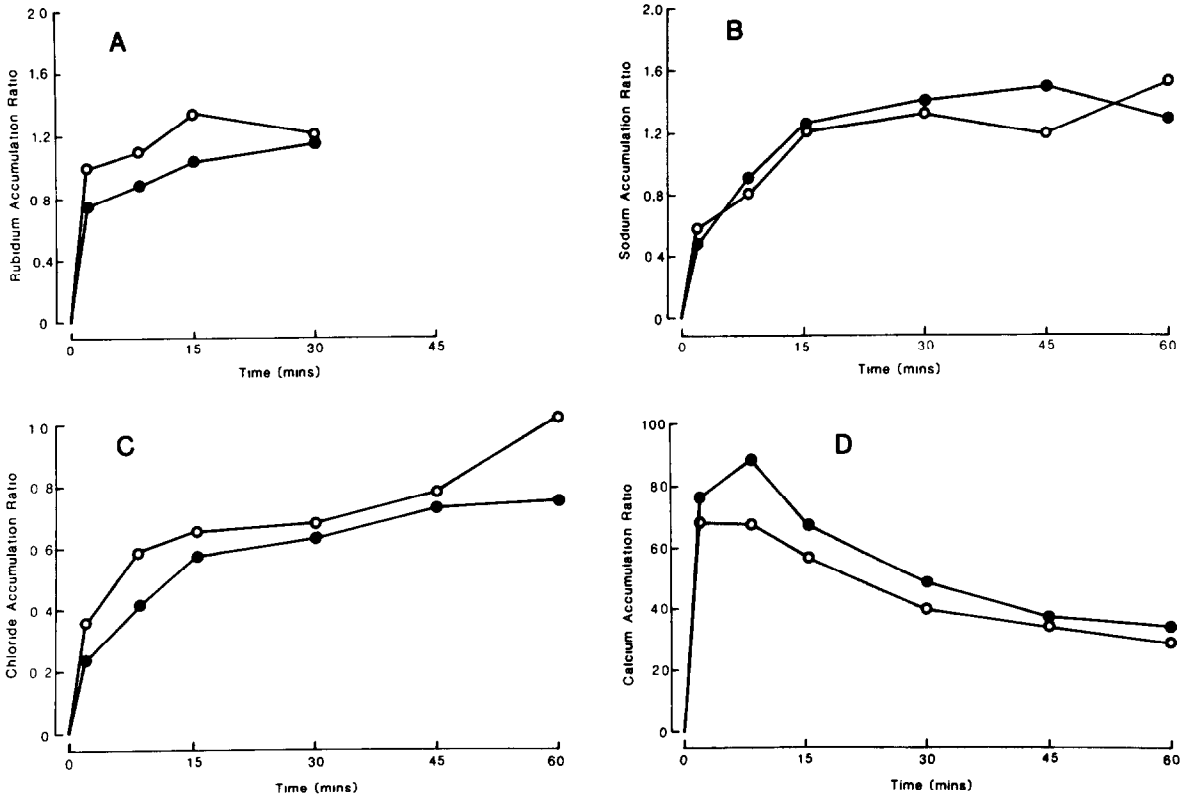

Fig.2. Uptake of ions into disks. ROS were incubated in light (open circles) or darkness (closed circles) in medium A with 1 mM ATP, 0.5 mM GTP, 1 μM TPMPCl, 0.1 mM inulin as in fig.1. Various combinations of $^{86}Rb^+$ or $^{22}Na^+$ or $^{36}Cl^-$ or $^{45}Ca^{2+}$ and ^3H_2O or [^{3}H]$TPMP^+$ or [^{3}H]inulin were used in different experiments. Disk volumes were always measured in parallel. The following key gives the panel, the labelled ion added and the (number of experiments): A, 0.2 μCi/ml ^{86}RbCl (2); B, 0.1 μCi/ml ^{22}NaCl (2); C, 0.2 μCi/ml Na^{36}Cl (3); D, 0.2 μCi/ml $^{45}CaCl_2$ (3). Each point was estimated in quadruplicate in each experiment.

Table 1

Ion balances in disks

	$TPMP^+$		$Rb^+(K^+)$		Na^+		Cl^-		Ca^{2+}	
	D	L	D	L	D	L	D	L	D	L
AR_x	12.8	18.1	1.01	1.09	1.42	1.45	0.53	0.69	59.1	55.6
SD	1.4	2.2	0.14	0.12	0.17	0.15	0.04	0.05	5.0	4.1
$[X]_{in}$	—	—	92.9	100.3	28.4	29.0	10.6	13.8	2.96	2..78
E_x	—	—	0.3	2.2	9.0	9.5	16.3	9.5	52.3	51.5
S.L.	0.001%		n.s.		n.s.		2%		1%	

Summary of data obtained from measurement of disk ion uptake as in figs 1 and 2 at 15 min after start of incubation. D, dark; L, light; AR_x, accumulation ratio of ion X; SD, standard deviation of Ar_x. $[X]_{in}$, concentration (mM) of ion X inside disks if all were free in solution; E_x, equilibrium potential (mV) of ion X if all is free inside the disks; S.L., significance level of difference between light and dark conditions calculated using Wilcoxon's signed-ranks test on all paired data from all experiments and all estimates. Disk volumes were measured in parallel in all experiments. Values are from quadruplicates on at least 7 preparations

Panel C of fig.2 shows that the $^{36}Cl^-$ accumulation ratio reached a plateau at about 0.6 and equilibrated with a similar time course to that of $^{22}Na^+$. Again swelling experiments supported the conclusion that the disk membrane is less permeable to Cl^- than K^+ (not shown).

Fig. 2D shows that the $^{45}Ca^{2+}$ accumulation ratio rises rapidly and then decays. Whether this accumulation is due to binding of $^{45}Ca^{2+}$ to the disk membrane or to a genuine concentration difference across the membrane is unclear. The decay indicates that net Ca^{2+} is leaving the pelleted material.

From fig.2 it appears that light causes small differences in accumulation ratios. However, owing to the difficulty of obtaining large quantities of material and the low intradiskal volumes it proved impossible to perform all the experiments in parallel on the same day. Consequently, results presented are averages from several experiments. The day to day variations were larger than the light/dark differences and so standard deviations on each point make the differences appear insignificant. However if a paired sample statistical test is applied (such as Wilcoxon's) to determine whether a consistent light/dark difference is seen it is found that the differences in fig.1 and fig.2C and D are significant whilst those in fig.2A and B are not. Table 1 shows an example of this detailed analysis performed for the 15 min time points of figs 1 and 2. Thus we can conclude that light induces an increase in disk V and Cl^- content and a decrease in disk Ca^{2+} content occurring within 10 min of the start of illumination.

4. DISCUSSION

Clearly, the time scale and the light exposures used to not correspond to those of interest in vivo but these observations do define properties of disks which may be of use in future studies.

A 30 mV potential across the disk membrane must be generated by some balance of ion gradients and permeabilities, assuming no steady energy-consuming pump is in operation. Table 1 shows that only Ca^{2+} or Cl^- of the ions we have studied could have set the potential at such a high value. Since the disk membrane is less Cl^- permeable than K^+ permeable this ion cannot be responsible. Consequently Ca^{2+} is the only ion which could. Whether in fact it does is unresolved.

In darkness, at early times when V is below 10 mV we can rule out Cl^- as responsible for setting V but it is not possible to decide between the other ions measured. However, in view of the large K^+ permeability and high K^+ concentrations, it seems possible that the K^+ accumultation ratio of about 1 clamps V close to 0 mV.

After prolonged incubation V begins to rise even in disks kept in the dark. Similarly the gradual loss of disk Ca^{2+} occurs in darkness but with a lag behind the trend in light. The reasons for this are not understood.

We endeavoured to perform our experiments under physiological conditions as far as possible. We adopted the media and regime used by George and Hagins [17] under which they observed light induced Ca^{2+} release from disks. Our results are consistent with theirs. Potential changes observed in isolated disks under non-physiological conditions (in particular with no Ca^{2+} present) are not inconsistent with our results [27–31]. Various studies of disk membrane permeability have been made. Uhl et al. [12] found disks to be most permeable to K^+ in darkness whilst studies of the effect of light have, unlike our work, revealed non-specific permeability increases [22–24]. We see no reversibility of the effect of light. This may be due to the unphysiological bleaches of the damaged state of the ROS. If Ca^{2+} release does occur in vivo there must be a re-uptake system as yet unknown. This may be affected by cyclic GMP [17,34].

We conclude that the disk membrane has little membrane potential in darkness but that on illumination a 30 mV potential is generated, possibly by an increase in disk Ca^{2+} permeability. This is accompanied by a loss of Ca^{2+} and gain of Cl^- by the disks.

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